## CLAIMS

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A bacterium attenuated by a non-reverting mutation in each of the aroC gene, the ompF gene and the ompC gene.

- 2. A bacterium according to claim 1 which infects by the oral route
- 10 3. A bacterium according to claim 1 which is from the genera Escherichia, Salmonella, Vibrio, Haemophilus, Neisseria, Yersinia, Bordetella or Brucella.
- 15 4. A bacterium according to claim 3 which is a strain of Escherichia coli, Salmonella typhimurium, Salmonella typhi, Salmonella enteritidis, Salmonella choleraesuis, Salmonella dublin, Haemophilus influenzae, Neisseria gonorrhoeae, Yersinia enterocolitica, Bordetella pertussis or

Brucella abortus.

5. A bacterium according to claim 4 which is a strain of enterotoxigenic E.coli (ETEC).

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6. A bacterium according to any one of the preceding claims which is further attenuted by a mutation in a fourth gene.

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- 7. A backerium according to claim 6 wherein the fourth gene is aroA, aroD, aroE, pur, htrA, galE, cya, crp, phoP or surA.
- 5 8. A bacterium according to any one of the preceding claims, wherein the mutation in each gene is a defined mutation.
- 9. A bacterium according to any one of the preceding claims, wherein the mutation in each gene is deletion of the entire coding sequence.
- 10. A bacterium according to any one of the preceding claims which has been genetically engineered to express a heterologous antigen.
  - 11. A bacterium according to claim 10, wherein expression of the antigen is driven by the nirB promoter or the htrA promoter.
  - 12. A vaccine comprising a bacterium as defined in any one of the proceding claims and a pharmaceutically acceptable carrier or diluent.
  - 25 13. A bacterium as defined in any one of claims 1 to 11 for use in a method of vaccinating a human or animal.
  - 14. An enterotoxigenic E.coli cell attenuated by a non-reverting mutation in each of the aroC gene, the



omp gene and the ompC gene, for use in a method of vaccinating a human or animal against diarrhoea.

- 15. Use of a bacterium as defined in any one of claims

  1 to 11 for the manufacture of a medicament for vaccinating a human or animal.
- 16. A method of raising an immune response in a mammalian host, which comprises administering to the host a bacterium attenuated by a non-reverting mutation in each of the aroC gene, the ompF gene and the ompC gene.

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